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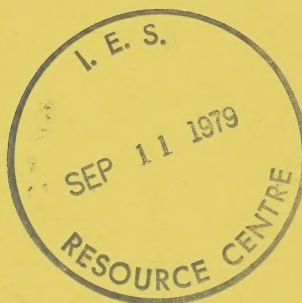
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VIRUSES IN THE ENVIRONMENT AND THEIR POTENTIAL HAZARDS

INST. FOR ENVIRONMENTAL STUDIES
UNIVERSITY OF TORONTO



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NOV. 14, 1973

CANADA CENTRE FOR INLAND WATERS
BURLINGTON, ONTARIO



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P R O C E E D I N G S

OF

SYMPOSIUM

VIRUSES IN THE ENVIRONMENT AND
THEIR POTENTIAL HAZARDS

WEDNESDAY, NOVEMBER 14, 1973

9:30 AM - 5:30 PM

CHAIRMAN: DR. GERALD BERG

MODERATOR: DR. M.S. MAHDY

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CANADA CENTRE FOR INLAND WATERS

MORNING SESSION

Chairman: Dr. Gerald Berg

Moderator: Dr. M.S. Mahdy

0930

Mr. J.P. Bruce
Director
Canada Centre for Inland Waters

Welcome

0935

Dr. M.S. Mahdy
Consultant
Microbiology Laboratories
Canada Centre for Inland Waters

Program Introduction

0945

Dr. Gerald Berg
Chief of Virology
Advanced Waste Treatment
Research Laboratory
Environmental Protection Agency
Cincinnati, Ohio

"Current status of viruses in
the environment and their
potential health hazards"

1010

Dr. E.L. Medzon
Associate Professor of
Bacteriology and Immunology
University of Western Ontario
London, Ontario

"Efficiency and limitations
of sampling and detection
techniques of viruses in
water"

1035 - 1055

COFFEE

1055

Dr. Donald M. McLean
Professor and Head
Division of Medical Microbiology
University of British Columbia
Vancouver, British Columbia

"Human enteroviruses in water
and wastewater and their
implications"

1120

Dr. J.W. Mosley
Director
Hepatic Epidemiology Laboratory
Liver Service, John Wesley Hospital
Los Angeles, California

"Water-borne viral hepatitis"

1145 - 1230

PANEL DISCUSSION

1240 - 1400

LUNCH

VIRUSES IN THE ENVIRONMENT AND
THEIR POTENTIAL HAZARDS

PERSPECTIVE

M.S. MAHDY

The objective of this symposium is to review our knowledge regarding the implications of the presence of human and animal viruses in both the air and water environment. Because of the possible causal relationship of viruses to malignant disease in man, the effect of viral pollution of waters on the frequency of tumors of fish and on viral carcinogenesis in this form of life is also sought. A corollary requirement in such a review, is a discussion of the methods used for the detection of viruses in air and water and of the methods used for both their removal and inactivation.

Through this approach, a picture of the potential hazards of viruses in the environment can be seen. Direction of research can then be affected. If potential hazards do exist, we should consider the implementation of monitoring programs, the revision of water quality standards to include viruses and the initiation of appropriate preventive measures. The following discussion is limited to some aspects regarding human viruses in the water environment.

Man is the reservoir of a large number of viruses that can be present in the water environment. These viruses are resistant to acidity of the stomach and to bile salts, are excreted in the feces and/or urine for prolonged or relatively prolonged periods of time, are stable under environmental conditions and spread usually via the oral-fecal route. Human viruses that possess such characteristics include the enteroviruses, adenoviruses, reoviruses, type A hepatitis and possibly the agent(s) of infectious non-bacterial gastroenteritis. The recent demonstration of appreciable amounts of hepatitis B antigen in feces

should provoke studies for the detection of this antigen in sewage and in effluents. Need for such information is stressed because the role of non parenteral routes in the transmission of type B hepatitis virus is more common than previously assumed.

Some other viruses such as plantar warts and molluscum contagiosum can be, and have been, reported to be transmitted through, or linked to, swimming pools.

Control of poliomyelitis has been widely successful through vaccination. Vaccines effective against the rest of the above viral agents are either unavailable, not practical or have limited use.

Large numbers of different viruses are present in raw sewage, in effluents and in streams receiving effluents. Calculated virus flow was reported in the order of about $10^5 - 10^6$ plaque forming units (PFU) per minute. This level of contamination is significantly reduced through dilution and hazardous levels of about 70 PFU per 50 gallons of river water have been demonstrated. Furthermore, possible contamination of potable water with viruses cannot be entirely excluded.

Viruses circulating in sewage represent a pooled sample collected from a community. They reflect the current pattern of infections with enteroviruses and probably with adenoviruses and reoviruses. This pattern may even be recognized before clinical cases are reported.

With the exception of unusual technical failures, it is unlikely that major water-borne viral outbreaks will occur under the present sanitary regulations, particularly in the developed countries. The danger involved is that of low level water-borne viral transmission resulting in scattered viral infections and in the introduction of viruses to communities that may have a large pool of susceptible individuals. The primary case(s) may occur through the environmental route, then through person to person contact a large number of secondary cases can occur. Epidemiological investigations of the relationship between surface water and disease are further complicated by the predom-

inance of asymptomatic infections, which are only recognized by laboratory tests, over apparent clinical disease.

The uniformity of clinical signs and of incubation period, increased incidence of cases, and uncommon seasonal or geographic distribution of viral disease may raise the possibility of a common vehicle and a common infective agent associated with these cases. The wide spectrum, however, of disease manifestations caused by enteroviruses and adenoviruses may not facilitate the recognition of such possibilities. That different viruses may produce the same syndrome and that the same virus may produce a variety of syndromes add to the complexity of the situation.

Although costly and involving many difficulties, studies on the epidemiology of viral infections in downstream communities receiving water or effluents with known spectrum of viruses should prove quite informative. These types of investigations require prior recognition of the immunological experience in the community. Thus, determination of the population at risk for specific viral infections can be made.

The use of coliform counts as an index of the presence or absence of fecal contamination can be a guide line, but has limited use with regard to viruses. Viruses have been demonstrated in waters in the absence of coliforms and poliovirus has been shown to be more resistant to chlorination than *E. coli*. The rapid recognition of the presence of viruses and the assessment of their removal and inactivation is necessary in any viral monitoring program. Unfortunately, the techniques for the detection of human viruses are time-consuming and expensive and there is need for indicators of viral contamination. Recently it was demonstrated that the f₂ phage, a ribonucleic acid phage with similar properties to poliovirus, is more resistant to chlorination than poliovirus. Therefore, under similar conditions, inactivation of this phage by chlorination indicates the inactivation of poliovirus but the survival of the phage is no definite proof of the survival of poliovirus. The search for such phages

or other similar microbial models with comparable biological and biophysical properties to viruses contaminating the environment may yield at least some tentative indicators of viral pollution. Such indicators can be detected rapidly, practically and economically and can be used if absolutely safe for seeding water or sewage to measure the efficiency of treatment processes.

The detection of small amounts of viruses in large volumes of water has been possible through a variety of concentration techniques. The quantitative efficiency of virus recovery, however, vary within a wide range among the different methods and sometimes within the same method. Standardization of these techniques and the reproducible demonstration of their sensitivities by separate laboratories are needed. Failure to recover viruses from waters may reflect lack of the sensitivity of the methods employed for the detection of the existing levels of contamination rather than the absence of viral agents in the samples tested.

The standards of water quality proposed in the U.S.A. of one detectable infectious virus unit per 10 gallons of recreational water and of one unit per 100 gallons of drinking water can be accepted as tentatively but not as absolutely safe. These standards should not be evaluated from the viewpoint of minimizing the health hazard by asking: who will drink 10 gallons of water to ingest one infective virus unit? Any individual will drink, over a period of time, 10 gallons of water and may ingest one unit. The question should rather be: what is the frequency of susceptible individuals ingesting one infectious unit and contracting infection? These frequencies would determine the probable number and magnitude of scattered infections that can easily be spread via person to person contact. Our estimates of the minimum infective dose for man are based on experimental models under controlled, but not natural, circumstances. Under various environmental and biological conditions man may be a more sensitive host for the detection of viruses than cultured cells.

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CURRENT STATUS OF VIRUSES IN THE ENVIRONMENT
AND THEIR POTENTIAL HEALTH HAZARDS

GERALD BERG

Virtually all domestic sewage contains large numbers of some of the more than one hundred different viruses that infect man and are excreted with his feces. Although many treatment procedures (even settling alone) are capable of removing large quantities of viruses from sewage, no process is capable of removing them all, and effluents discharged into rivers and streams usually carry with them large numbers of viruses that become a potential hazard to recreationalists and to downstream communities that must potabilize these waters. Viruses have been detected at water intakes many miles downstream of outfalls even with the relatively insensitive methods presently available for recovering small amounts of viruses from large volumes of water.

Effluents containing appreciable quantities of solids are at best rarely, and possibly never, disinfected completely by chemical means, because it is unlikely that such agents can enter into the fecal particles of which viruses may be an integral part. Unless heat or ionizing radiation is the disinfecting agent, inactivation of all viruses in an effluent may well depend upon the prior removal of substantially all solids and all dissolved substances from those effluents that would interfere with the disinfectant. For effluents and raw waters alike, the disinfectant of choice is always one tailored to the need, and the best primary indication of microbiological quality is the accurate in situ measurement of the disinfecting chemical species (a goal yet to be attained) since waters are usually long gone before bacterial and viral indicators can be measured.

Bacterial indicators of fecal pollution, useful as they may be, are not always present (in standard tests) when viruses are recovered. Thus, it is important to improve bacterial

indicator systems and to develop quantitative methodology for recovering small numbers of viruses from large volumes of effluents and other waters. Moreover, since most viruses present in water are often adsorbed to solids, it is important to develop quantitative methodology for recovering viruses from the fecal and other solids present in effluents and waters. With the rapidly growing interest in disposal of sludges to the land, it becomes commensurately important, also, to develop quantitative methodology for detecting viruses in sludges.

The great concern for viruses in the water environment derives from the capability of even the smallest quantity of viruses that we can detect by the most sensitive methods we have available to infect man. Unfortunately, as dramatic as the available data are, they are derived from limited experiments in man under conditions that bring the virus more frequently in contact with susceptible cells than is likely in the normal consumption of waters contaminated with small amounts of viruses. Although available experimental data demonstrate clearly the potential hazard of consuming virus-bearing waters, the quantitative risk is yet to be determined.

Epidemiological support for water transmission of viruses exists only for infectious hepatitis. Such evidence in support of transmission of other viruses by the water route may have been confounded in the past by the neglect of infection in favor of disease induction as the indicator of such transmission.

There is also now accumulating slowly evidence that some tumors of fish are associated with certain sewage outfalls. If chemical agents capable of inducing latent viruses should be implicated in such tumors, or if viruses per se should be implicated directly, the inferences to the downstream consumer of water would be clear.

NOTES

EFFICIENCY AND LIMITATIONS OF SAMPLING AND DETECTION
TECHNIQUES OF VIRUSES IN WATER

E.L. MEDZON

This rather pretentious title can be reduced to two aspects; 1. What do we want to know when we detect viruses in the environment, their presence or their infectivity? 2. How do we sample enough water so that the usual low numbers of viruses can be concentrated to be useful for our detection techniques?

Viruses represent a class of microbial agents which are unique as intracellular parasites. They are very small (from 17 nm* up to 800 nm), are greatly varied in shape (from icosahedra to long filaments, with complex construction in many forms), contain varying amounts of either deoxyribonucleic acid (DNA) ribonucleic acid (RNA), have varied but usually specific hosts, are serologically distinct, and require an intracellular environment for their replication because they have no energy synthesizing mechanism and very few, if any, enzymes. They are varied in their response to the environment in the cell free state and may be either inactivated naturally and unable to replicate when a suitable host cell is provided or very resistant to inactivation processes. Therefore the detection of viruses rests on the ability to concentrate selectively these particles from water in order to get enough material to test for their presence.

Concentration methods have been devised for animal and microbial viruses which utilize adsorption onto gauze pads (efficiency about 15%) with great success in sewage (11-88% isolation rate) but this method is regarded as an unuseful procedure in finished or surface water unless a flow through device is used. Adsorption on activated charcoal, polyphosphate, iron oxide or ferric chloride has been used to remove viruses from water, but recoveries for detection vary. Calcium phosphate and aluminum hydroxide gels have been used with some success (80-100%

*nm - nanometer = 10^{-6} millimeter

recovery), as has salmine precipitation (80-100% recovery). The introduction of anion exchange resins or polyelectrolytes of known chemical composition has shown high efficiency of virus adsorption and elution (close to 100% and 12-80% respectively) and is applicable to large volumes (100 or more litres). These procedures usually require that particulate material be removed by prefiltration, and they can be modified to a one-step procedure if a filter "sandwich" is used.

Other physical methods in use include two-phase polymer extraction (35-100% recovery) and those in which water is removed by osmotic ultrafiltration (90-100% recovery), electrophoresis and electroosmosis, and hydroextraction, but these methods appear to be useful only for smaller volumes and may require special equipment. Filtration through alginate filters works well with smaller volumes which are relatively clean (25-100% recovery). A major method in current use is the adsorption of viruses onto cellulose ester filters. These membranes can be very efficient (25-100% recovery) depending on the care taken to modify pH and ionic strength for adsorption and elution, and can be incorporated into large scale flow through systems.

Lastly, centrifugation of viruses in an ultracentrifuge can be applied to large volumes by the use of continuous flow rotors. Isopycnic or rate zonal density gradient centrifugation can be used to isolate the particles of interest with 80-90% recovery, but the expense involved makes it prohibitive for most laboratories.

A new approach which should be considered is the use of immunoadsorbents to isolate selectively viruses on the basis of their surface antigens.

The animal viruses which are considered to be typically found in water are the enteroviruses (Picorna) e.g. poliovirus, coxsackievirus, echovirus, reoviruses, and adenoviruses, although other viruses may be present. They are detected by infectivity assays, usually in tissue culture, by a quantal (dose response) or enumerative (infectious particle count) method. These assays

assume that the infectivity of a sample represents the potential content and hazard of water. An argument can be made that there are more non-infectious, than infectious particles in any sample, and these can be used to increase the sensitivity of virus detection and identification. Furthermore, viral nucleic acid may still be infectious although the particle may be unable to infect a cell. The use of regular and immunoelectronmicroscopy, radioactive immune assays and immunocytological techniques as adjuncts to infectivity assays on concentrated samples should increase the detection sensitivity of methods of and give a broader view of the viral content of water.

NOTES

HUMAN ENTEROVIRUSES IN WATER AND WASTEWATER
AND THEIR IMPLICATIONS

D.M. MCLEAN

Enteroviruses have been associated repeatedly with widespread outbreaks and sporadic cases of human illnesses including aseptic meningitis, pleurodynia, pericarditis, neonatal myocarditis, serous peritonitis, maculopapular or vesicular exanthemata, undifferentiated febrile disorders (summer gripe), some acute respiratory infections such as coryza, acute laryngotracheobronchitis or undifferentiated upper respiratory illness, and occasionally with infantile gastroenteritis. The enteroviruses comprise over 30 serotypes of coxsackievirus, almost 40 echovirus serotypes, plus three serotypes of poliovirus, one of which caused a small epidemic of paralytic poliomyelitis in a Connecticut population group during September 1972. All of these viruses have been isolated from the human alimentary tract, and in many instances from other organs and fluids.

Enteroviruses are excreted into the environment principally through the feces. Thus they may contaminate water readily through ineffective methods of sewage disposal.

Since methods for detection, identification and titration of enteroviruses are rapid and convenient for most virological laboratories, several avirulent strains have been used to investigate the dispersal and inactivation of these agents in natural waters. Criteria, which denote the quantitative relationships between the virus pollutant in water and human infection, have been developed for a variety of natural and domestic situations. To date, all depend on the ability to detect as small a quantity as 1 TCD₅₀* per ml virus. However the standard of complete absence of enterovirus infectivity from water can be attained only by the development of new techniques for detection of trace quantities

*TCD - tissue culture infective dose

(possibly 0.01 TCD₅₀) in large volumes (possibly 1 litre) of water which may be contaminated with other chemical or microbiological pollutants.

The minimum dose of the Fox strain of attenuated poliovirus-3 which infected 50% of infants by feeding was calculated to be 4 TCD₅₀. Upon feeding the SM strain of attenuated poliovirus-1 in gelatin capsules to adult human volunteers it was calculated that 50% became infected by 1 PFU**. Feeding of 10⁵ TCD₅₀ of P-712 Sabin attenuated poliovirus-2 regularly induced alimentary infections in unvaccinated volunteers, but feeding of 10⁵ TCD₅₀ to a vaccinated subject was not followed by virus excretion in feces. Previously unvaccinated men have not become infected by swimming in freshwater containing 1 to 5 TCD₅₀ per ml. No experimental data are currently available regarding the minimum infective doses for humans of nonpolio enteroviruses. Since ingestion of 1 litre of water with an enterovirus concentration of 1 TCD₅₀ per ml could introduce 10³ TCD₅₀ into a subject during swimming or thirst-quenching, a non-immune human could readily receive an infectious dose. Whether he develops illness subsequently depends upon the ratio between clinical illness and inapparent infections, which for poliovirus is usually below 1:100.

Enteroviruses are dispersed in natural waters according to the rate of flow or turnover of water plus surface movement by wind or mechanical agitation. Their viability is inactivated rapidly by residuals of free chlorine or other halogens, and more slowly by sunlight, aeration, seawater and miscellaneous chemical contaminants. Their concentration may also be reduced by adsorption to sand. Examples of enterovirus prevalence and inactivation will be discussed regarding drinking water, freshwater in streams and lakes, seawater in estuaries and along open beaches, and wastewater before and after treatment, including notes on virus detection in water toxic for tissue cultures.

**PFU - plaque forming unit

NOTES

WATER-BORNE VIRAL HEPATITIS

JAMES W. MOSLEY

Epidemics of type A hepatitis due directly or indirectly to contamination of water continue to be reported. In recent years in the United States and in Canada, however, they involved only private supplies, usually in obvious violation of established standards. Such occurrences contribute a negligible proportion of total cases. In developing countries, regular supplies for drinking are more frequently contaminated, and water-borne infection sometimes contributes substantially to total morbidity. In all areas of the world we must guard against the possibility of major epidemics because of unexpected natural occurrences or human error.

Endemic water-borne type A hepatitis, other than indirect transmission through raw shellfish, has not been documented. Attempts to do so cannot be regarded as successful. In the United States and Canada, a declining incidence of type A hepatitis, in the face of increasing pollution, argues against any significant role of water in the ecology of hepatitis A virus.

Type B hepatitis must now be considered as potentially water-borne in view of the finding of hepatitis B antigen in one sample of oysters from a bed proximate to a coastal hospital's sewage outfall. The presence of hepatitis B virus in feces has yet to be demonstrated, and infectivity by mouth is of a much lower order of magnitude than by the percutaneous route. The problem, therefore, seems minimal, but evaluation is certainly appropriate.

NOTES

AFTERNOON SESSION

Chairman: Dr. Gerald Berg

Moderator: Dr. M.S. Mahdy

1400

Dr. J.B. Derbyshire
Professor
Department of Veterinary
Microbiology and Immunology
University of Guelph
Guelph, Ontario

"Viral pollution hazards of
animal waste"

1425

Dr. Otis Sproul
Professor of Civil Engineering
University of Maine
Orono, Maine

"Effectiveness of water and
wastewater treatment processes
in virus removal and
inactivation"

1450

Dr. N.St. G. Hyslop
Head of Immunology
Animal Pathology Division
Health of Animals Branch
Canada Department of Agriculture
Animal Diseases Research Institute
Hull, Quebec

"Contamination of the environ-
ment by airborne filterable
organisms"

1515 - 1535

COFFEE

1535

Dr. Eric Brown
Professor and Chairman
Department of Microbiology
Chicago Medical School
Chicago, Illinois

"Incidence of tumours in fish
in polluted and nonpolluted
water systems: viral
implications"

1555

Dr. R.S. Sonstegard
Research Scholar
Department of Microbiology
University of Guelph

"Relationship between environ-
mental factors and viruses in
the induction of fish tumours"

1615

Dr. J.C.N. Westwood
Professor and Head
Dept. of Microbiology & Immunology
University of Ottawa
Ottawa, Ontario

"Environmental transmission
of viral infections"

1640 - 1720

PANEL DISCUSSION

1720 - 1730

Dr. M.S. Mahdy

Summary and Future Prospects

VIRAL POLLUTION HAZARDS OF ANIMAL WASTE

J.B. DERBYSHIRE

Although environmental pollution with viruses of human origin is fairly well recognized, particularly in the form of water pollution with viruses from domestic sewage, much less is known about the viral pollution hazards of animal wastes. In this paper, attention will be devoted to a consideration of the possible contribution which farm livestock wastes may make to viral pollution of the environment.

Animal wastes which may constitute a viral pollution hazard include the secretions and excretions of livestock, together with litter which may be contaminated with these materials, in addition to infected carcasses and various abattoir wastes. Of these potential sources of pollution, only abattoir wastes have received any significant investigation, and these only on a limited scale. Although only clinically healthy animals are slaughtered for human consumption, these may well be carriers of virus at the time of slaughter, and potentially contaminated products such as intestinal contents represent a disposal problem. In limited investigations, animal viruses have been detected in abattoir waste water.

The presence of animal viruses in carcase waste products is a rather well recognized hazard in veterinary medicine. Thus foot-and-mouth disease, vesicular exanthema and vesicular disease of swine have all been shown to spread when swine have been fed unsterilized garbage infected with these viruses.

Increasing intensification of the livestock industry has increased the magnitude of problems associated with the safe handling of animal wastes. Large concentrations of animals in single units, such as beef cattle feedlots and intensive dairy, swine and poultry operations produce massive local accumulations

of infected material which present a major disposal problem. In order to reduce labour costs, the handling of excreta in liquid form has become popular in recent years, and the volumes of slurry or liquid manure which may be involved may force operators to dispose of such materials in an unsafe fashion, whereas solid manure could more readily be stacked and composted before disposal on agricultural land.

Animal viruses in liquid or inadequately composted solid manure which is spread on agricultural land may be a source of widespread environmental pollution. Where pasture land has to be used for this purpose, direct infection of grazing animals may result. Contamination of surface water may occur by means of storm water run-off from agricultural land, and contamination of ground water supplies is a possibility under certain circumstances. Transmission of viral infection by the water route to man and other animals may be associated with a contaminated water supply when the latter is inadequately treated. In addition, during the disposal of manure on agricultural land, aerosols containing viruses may be generated, and there may be airborne transmission of infection to man and animals. The viruses of foot-and-mouth disease and Newcastle disease have been shown to be capable of airborne spread over distances of several miles.

The main contribution to the viral content of solid and liquid manure is from the intestinal tract of animals. In cattle, the recognized enteric viruses include enteroviruses, reoviruses and other diplomnaviruses, parvoviruses, coronaviruses and mucosal disease virus. In swine there are enteroviruses, reoviruses, adenoviruses, parvoviruses and coronaviruses, and in sheep the isolation of adenoviruses, enteroviruses and reoviruses from faeces has been recorded. Of these enteric viruses, the enteroviruses, adenoviruses, diplomnaviruses and parvoviruses are those most likely to be capable of survival for significant periods in the environment. Animal wastes may also be contaminated by viruses

excreted from the urinogenital and respiratory tracts, and from saliva and skin, although some of the viruses present in these situations are in general less well able to survive than those which infect the intestinal tract.

Of the viruses which are common to man and animals, certain of those which cause severe disease in man, such as rabies and some of the arboviruses, are unlikely to spread by the water route since a vector is normally required for their transmission. Others, such as the reoviruses, may be readily transmitted among animals and man in this way, and there may be additional viruses which are currently undetected which may represent an unrecognized hazard.

NOTES

EFFECTIVENESS OF WATER AND WASTEWATER TREATMENT PROCESSES
IN VIRUS REMOVAL AND INACTIVATION

O.J. SPROUL

Virus removal by water and wastewater treatment processes are controlled in part by rate processes. This implies that long treatment times are required to produce waters with zero virus levels. Unfortunately, economic considerations have caused us to forego the construction of these types of plants. It follows, therefore, that viruses will be present in the effluent if they are in the influent. This is particularly true where domestic sewage is being treated and also, but to a lesser extent, when contaminated surface water is used for domestic water supplies.

The paper can best be summarized by citing the concentrations of viruses to be expected in the effluents from various types of treatment systems. These data have been taken from literature citations on virus removals obtained in the various unit processes as they are customarily used in North America.

Detailed data will be presented on these systems in the paper. Other less commonly used treatment methods for both water and wastewater will also be discussed.

VIRUS CONCENTRATIONS IN EFFLUENTS FROM
DOMESTIC WASTEWATER TREATMENT PLANTS

Type of Treatment	Removal Expected %	Virus in Effluent* **PFU/liter
Primary Treatment		
Primary Sedimentation	0	
Chlorination	50	3,500
Secondary Treatment		
Activated Sludge	90	
Chlorination	50	350
Tertiary Treatment		
Precipitation of Phosphate and Sedimentation	90	
Activated Carbon Adsorption	10	
Chlorination	99	6

*Assumes 7,000 PFU/liter in the influent.

**PFU - plaque forming unit

WATER VOLUME TO CONTAIN ONE VIRUS (PFU)**
AFTER INDICATED TREATMENT*

Type of Treatment	Removal Expected %	Volume to Contain One Virus Gallons

Softening		
Excess Lime Precipitation	90	
Coagulation	90	
Filtration	90	
Chlorination	99.999	30,000,000
(FAC = 0.5 mg/l		
pH = 7.8		
Temperature = 0° - 5°C		
Contact Time = 30 minutes)		
 Filtration		
Coagulation	90	
Filtration	90	
Chlorination	99.999	3,000,000
(Same conditions as above)		
 Filtration		
Coagulation	90	
Filtration	90	
Chlorination	99	3,000
(Combined residual = 4.5 mg/l		
Temperature = 0° - 5°C		
Contact Time = 60 minutes)		

*Assumes 3 PFU/gal. on the raw water before treatment

**PFU - plaque forming unit

Future research and development efforts will probably take the following direction:

- (a) Protective effects created by virus clumping and/or adsorption to surfaces on the recovery, quantitation and inactivation of viruses.
- (b) Reduction of virus concentration procedures to practical techniques.
- (c) Infection and disease caused by low level virus transmission through the water supply.
- (d) Development of a virus standard for treated wastewater and drinking water.

NOTES

CONTAMINATION OF THE ENVIRONMENT BY AIRBORNE
FILTERABLE ORGANISMS

N. ST.G. HYSLOP

The possibility of airborne transmission of acute viral diseases, such as the common cold, influenza, measles, poliomyelitis and smallpox has been recognized for many years. Recently the list of airborne diseases of man has been enlarged greatly. Thus, there is some evidence that even disease agents, such as rabies virus and that of type B hepatitis, occasionally may be transmitted to new hosts by the air.

Many diseases of animals and poultry, notably bovine contagious pleuropneumonia, foot and mouth disease, rinderpest, infectious bovine rhinotracheitis, hog cholera, Newcastle disease, avian infectious bronchitis and Marek's disease are known to spread in the same way. In some dangerous or economically important maladies, airborne infection may play a more important role than ingestion or the contamination of wounds.

Numerous factors contribute to pollution of the environment by airborne viruses, mycoplasmata and bacterial L-forms. Probably the most frequent natural cause is exhalation of organisms (especially in coughs, sneezes etc.) following secretion into the respiratory tract during periods of viraemia. However, the atmospheric burden may be increased by release of other bodily secretions and excretions, by re-entrainment of contaminated liquid droplets or dust particles, by rainsplash, or by certain methods of sewage disposal. Special dangers exist when sewage from infected premises is transferred under pressure from one holding unit to another, when sewage or slurry tanks are opened, when atmospheric pressure changes result in sudden venting of inadequately protected sewage systems, or when untreated manure is sprayed onto pastures by apparatus such as "rain-guns". Poxviruses some herpesviruses and other organisms may be disseminated by incautious handling of bedding contaminated by epithelial

fragments. Vigorous sweeping of floor dust may be a particularly important source of local airborne infection.

Most naturally-generated aerosols consist of particles distributed within a wide range of sizes. The largest of these do not travel far before falling to the ground (where some of the more resistant organisms may remain infective for long periods in suitable ambient conditions). Smaller suspended infective dusts, droplets, microdroplets and droplet nuclei are removed from the atmosphere by impaction, sedimentation, electrostatic attraction to surfaces and also by their being washed from the air during periods of precipitation. The rate of "fall-out", and thus the degree of local contamination by relatively long-lived organisms, is enhanced under conditions which favour aggregation of particles. Particles which remain airborne for long periods are predominantly smaller than 5-10 microns in diameter. However, in still air at high relative humidity (RH) increase in size may occur as a result of coalescence, and this gradual increase in mean diameter is especially evident in aerosols of small initial size. Concentration and particle size is also determined partly by factors such as the nature of emission sources, topographic and meteorologic influences and by the density, hygroscopicity and mean shape of the infective material. Subsequently, modification of aerial distribution patterns result from changes in wind velocity or direction, thermal currents, altitude, weather and local turbulence. Inversions and down draughts may have a profound effect on deposition. Increased incidence of foot and mouth disease was observed on Swiss mountain ridges when prevailing winds travelled at 90° to the general axes of the valley systems, and down-draughts may be associated with similar concentration of infection as the result of "lee-wave" effects. On several occasions a new series of outbreaks of foot and mouth disease or of Newcastle disease has occurred in a new sector after a change in wind direction.

In the case of the filter-passing organisms, the rate

of "fallout" in "dry" conditions is determined much more by characteristics of the "carrier" particles (dusts, micro-droplets) and by the prevailing meteorologic conditions than by the sub-microscopic size of the agents themselves. Rainfall may remove significant amounts of infective particles from the air, to cause contamination of drinking water, crops and pastures. However, the relative importance of aerial or "ground" contamination will depend on the longevity of the organism, its mode of entry into the host, and whether it is more infective by ingestion or by inhalation.

Factors which influence the survival of airborne and, perhaps, recently deposited viruses include light (especially UV light), oxygen tension and RH. The effects of these factors are variably inter-related, depending on the particular organism, but RH usually exerts a very profound effect. In general, adenoviruses and some picornaviruses tend to survive better at high RH, whereas influenza, parainfluenza-3 and measles viruses survive better at lower RH. Contamination of airstreams may be greatest at night, when organisms are protected from the effects of insolation. Periods of nocturnal rainfall may cause increased contamination of surfaces. There is some indication that an undefined "open-air factor", possibly a chemical air pollutant, exerts an adverse influence on certain bacteria and viruses. At a reasonable distance from the source, the concentration of aerosols tends to be greater outside buildings than indoors, but there is some evidence that viruses persist longer indoors, where the sizes of solid aerosols are usually smaller and more uniform.

When only the respiratory route of infection is of epidemiological significance, particle size is of great importance, since it controls the site at which the greater part of the aerosol will be deposited after inhalation. Thus the larger particles, which tend to be removed in the upper respiratory tract, will be especially dangerous in diseases for which the portal of entry is the naso-pharynx, whereas particles of about

0.6 microns diameter will evoke a greater infection rate for viruses which gain access principally through the bronchioles and alveoli.

Detection of aerosols of filter-passing organisms presents special problems associated with the extreme fragility of many of the most pathogenic species, so that one may be compelled to infer their presence from that of certain "markers". Collection methods resemble the techniques employed for sampling other aerosols; each possessing inherent advantages and disadvantages. Electrostatic samplers appear to be lethal (ionization effects) for some viruses but not for others. "Porton impingers" (which may be arranged in parallel to sample large volumes) and microthread collectors prepared from spiders' webs are useful. The Lytton large-volume sampler has proved valuable for investigations on very large volumes of air under field conditions.

The ultimate significance of aerial dissemination of small infective agents depends partly on their longevity and partly on the presence in their locality of susceptible host populations or mechanical vectors. Furthermore, duration of viability in the airborne state may be dependent to a surprising degree on the species or the particular strain of the organism.

These considerations render forecasting of the extent of dissemination of a disease, and delineation of probable contaminated zones, except in very general terms, a matter of the greatest difficulty. The relative inefficiency of existing monitoring systems for biological materials leads to further caution regarding the feasibility of accurately predicting the aerial spread of viral diseases for more than a few miles beyond a primary outbreak.

NOTES

RELATIONSHIP BETWEEN ENVIRONMENTAL FACTORS AND
VIRUSES IN THE INDUCTION OF FISH TUMOURS

R.S. SONSTEGARD

During the course of these investigations questionnaires were circulated to biologists around the world inquiring as to the occurrence of neoplasia in poikilotherms. The responses to these questionnaires were both exciting and frightening. First, it is apparent that the occurrence of neoplasia in poikilotherms is no unusual phenomenon and that many "new" neoplasms were identified which may have value as models for human cancer research.

What is frightening, were the numerous reports of high incidences of neoplasia in poikilotherms inhabiting heavily polluted waters. These reports stimulate the following thought provoking questions. Do these tumours reflect the presence of carcinogens in the environment. Could they reflect the interaction of different chemicals in the environment? Oncogenic viruses and chemicals? Oncogenic viruses alone?

The potential utility of poikilotherms as sentinel animals for the early detection and identification of environmental carcinogens will be discussed with particular reference to the above considerations. The occurrence, epizootiology, geographical distribution, etc. of several epizootics of neoplasia in fresh water inhabiting polluted and non-polluted waters will be presented with particular reference to virus and induction of oncogenic viruses.

NOTES

INCIDENCE OF TUMOURS IN FISH IN POLLUTED AND NONPOLLUTED WATER
SYSTEMS: VIRAL IMPLICATIONS

ERIC R. BROWN

Last year we reported (Cancer Research 33:189, 1973) that polluted waters produced a higher frequency of fish tumours than control waters. In continuation of these studies, we found various tumour incidence to exist among fish species within the polluted system. The greatest differences (616%) were found among the brown bullhead Ictalurus nebulosus above and below the major points of contamination. The Fox River, our test river, revealed areas of heavy contamination with both organic and inorganic materials exceeding state and federal minimal safety standards. Laboratory tests revealed the presence of numerous viral agents, particularly near waste pipes near city storm sewer systems. These findings would implicate certain viruses and/or chemicals in the water as potential carcinogens for fish. Experiments were conducted on Esox lucius (Northern Pike). Interest centered around the possible viral origin of lymphosarcoma of the jaw in that species. Cell free filtrates of this tumour were inoculated into a test group of this species which had been hatched and raised in enclosed waters which were pollution free. One control group received an equal amount of a similar tissue extract of the lower jaw of normal fish. A second control group received Hanks balanced salt solution. The fish were separated in different tanks at all times. At the end of seven weeks the vast majority of fish inoculated with tumoured homogenates had developed lymphosarcoma, while no control fish developed tumours. These experiments strongly suggest the involvement of a filterable agent in this transmission.

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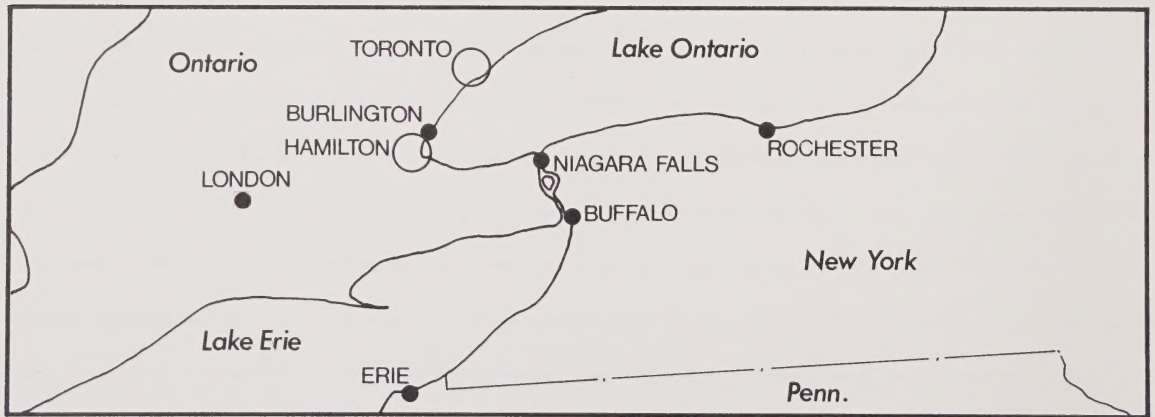
ENVIROMENTAL TRANSMISSION OF VIRAL INFECTIONS

J.C.N. WESTWOOD

The environment is a big place and a discussion of environmental factors in viral infection could be almost limitless in scope, but, in view of the purposes of this symposium will be confined to the possible significance of air and water in the epidemiology of virus disease. Because the environment is so big, the dilution factors involved are so great that the degree of contamination which can be produced, even deliberately, is necessarily very small in relation to the absorptive capacity of an individual human being. One million PFU* of virus dispersed into a cube of air only 100 yards on the side would result in a virus concentration of only one PFU/270 litres. At 10 litres/minute a man would have to breathe steadily for half an hour in order to inhale 1 PFU - and would then have 50% chance of exhaling it again. Since man's capacity for water is much less than that for air, the dilution factor is no less real in that context. The practical effect of contamination of the environment therefore must depend upon the issue of dosage. What is the infective dose for man of the various types of virus which may reach the water as a result of sewage pollution and the atmosphere as a result of aerosolization from water? What is the likelihood of an individual absorbing such a dose and what would be the consequences of his doing so? The issues of dosage are difficult and will be discussed in some detail in comparison with what is known for water-borne bacterial infections - particularly typhoid fever. That dosage may be adequate to make effective impact is undoubted as shown by the New Delhi hepatitis outbreak, but such episodes are rare and attention might well be paid to subtler effects on the human population. Here, too, comparison with the bacterial model suggests both similarities and differences which are interesting and may be informative.

*PFU - plaque forming unit

NOTES



HOW TO REACH
CANADA CENTRE FOR INLAND WATERS
867 LAKESHORE ROAD, BURLINGTON, ONTARIO

1. AIR

Toronto International Airport offers regular services by Air Canada, Allegheny, American, BOAC, CP Air, Eastern, Mohawk, North Central and United Air Lines. On arrival take the airport limousine service to the Ponderosa Motel, Burlington. This leaves the airport on the hour and the half hour; fare \$4.00. Local taxis are available from the Ponderosa to other motels or to the Canada Centre for Inland Waters.

2. TRAIN

Burlington station is a \$1.00 or \$2.00 taxi ride to motels or to the Centre.

3. CAR

(A) From Hamilton, approach Lakeshore Road (formerly Beach Blvd.) in Burlington from either east or west direction. The Canada Centre for Inland Waters is located beside the Burlington Canal on the east (or Burlington side) of the Skyway.

(B) Queen Elizabeth Way (Q.E.W.) from Toronto, follow the Niagara route and leave Q.E.W. just before approach to Skyway Bridge and take Lakeshore Road (formerly Beach Blvd.) to just before the ship-canal and lift-bridge. Turn right at well marked entrance.

(C) Queen Elizabeth Way from Buffalo and Niagara Falls, turn right off Q.E.W. just before toll gates to Skyway Bridge; turn left almost immediately onto Lakeshore road (formerly Beach Blvd) and continue to just beyond ship-canal and lift-bridge. Turn left at well-marked entrance.

FOR FURTHER INFORMATION CONTACT B.J. DUTKA AT 416-637-4286

